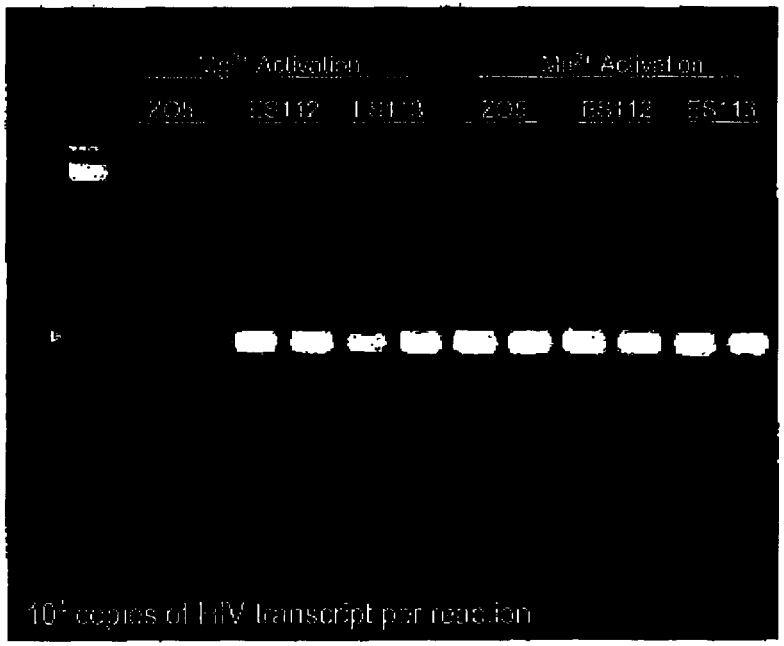
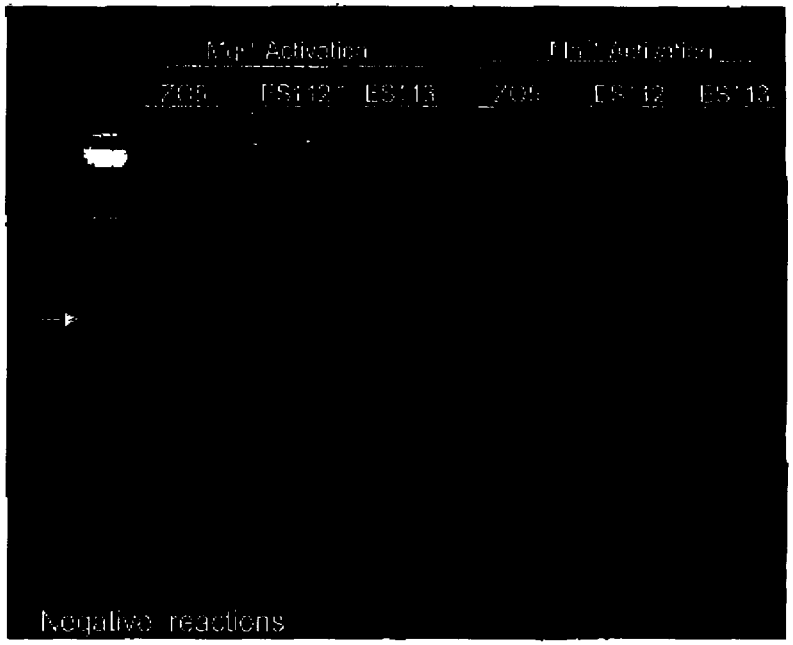
Improved Mg^{2+}-activated RT-PCR with ES112 & ES113

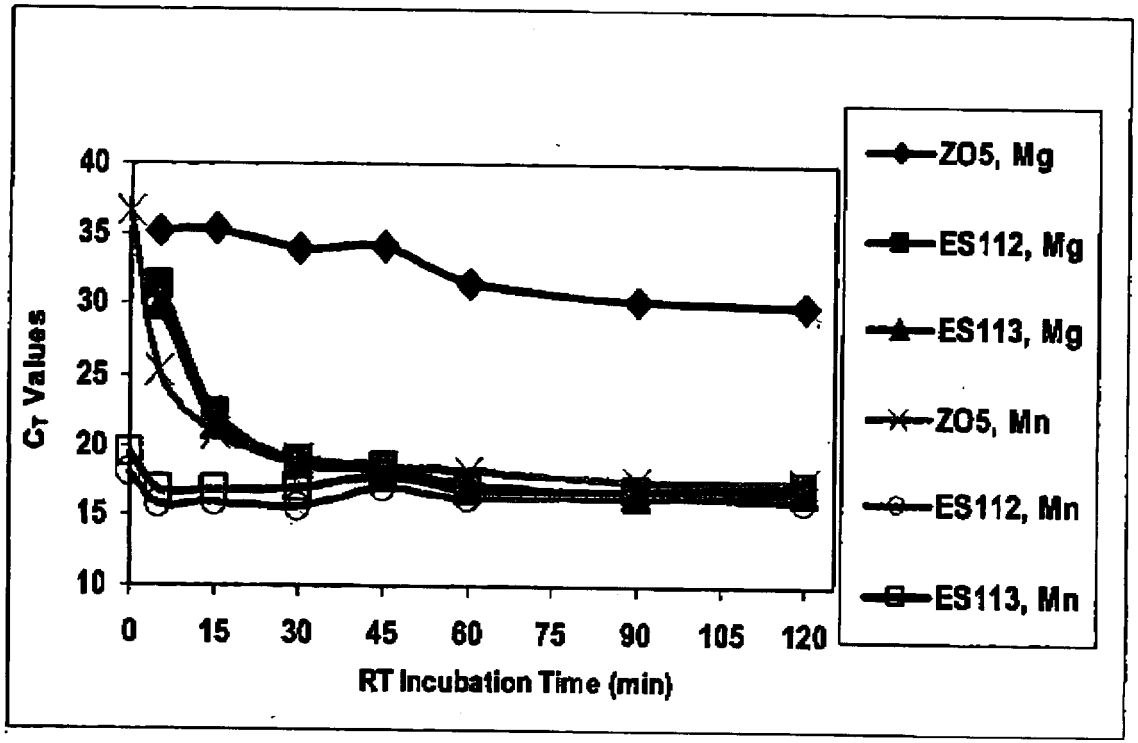




Three different thermostable DNA polymerases were used to reverse transcribe an HIV transcript RNA template and subsequently amplify the cDNA in a coupled RT-PCR in the presence of either 3 mM Mg^{2+} or 3 mM Mn^{2+}. After 55 cycles of PCR, gel results demonstrate specific amplification products from RNA with ZO5 in the presence of Mn^{2+}, but no specific product was observed when Mg^{2+} was used as the divalent metal ion activator. However, designer enzymes ES112 and ES113 produced specific amplification product with either Mg^{2+} or Mn^{2+} activation.

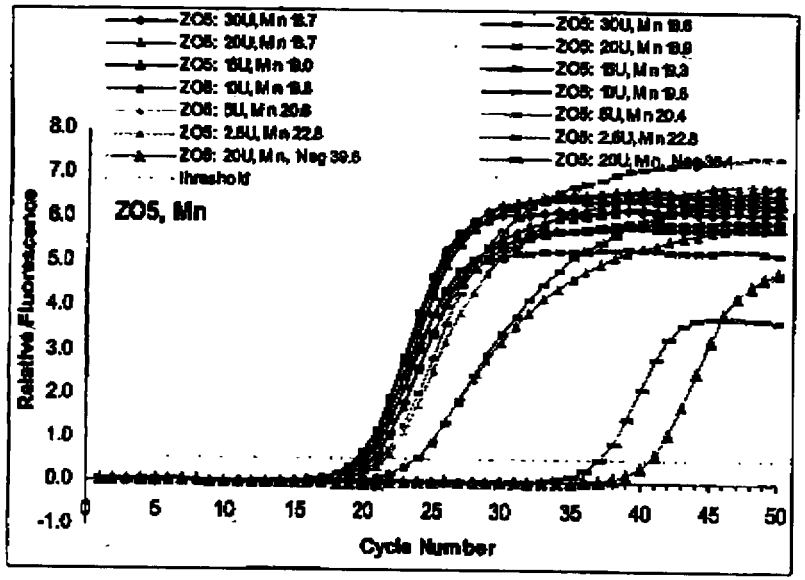
Reduced RT Time Requirement for ES112 & ES113 in Mn^{2+}

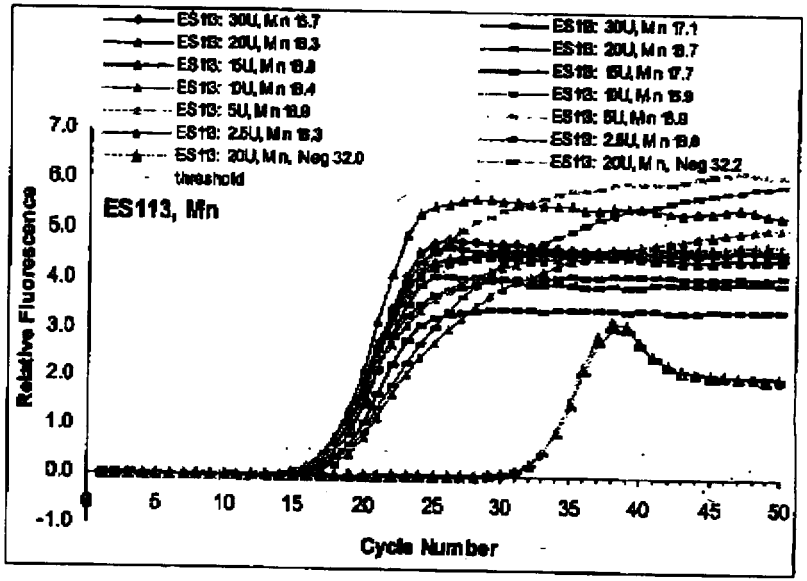
A 280 bp GAPDH RNA template was subjected to various RT incubation times and then amplified by PCR. In all cases PCR profiles were identical and the results were analyzed by kinetic PCR. The C_T values of growth curves are plotted in the following chart:

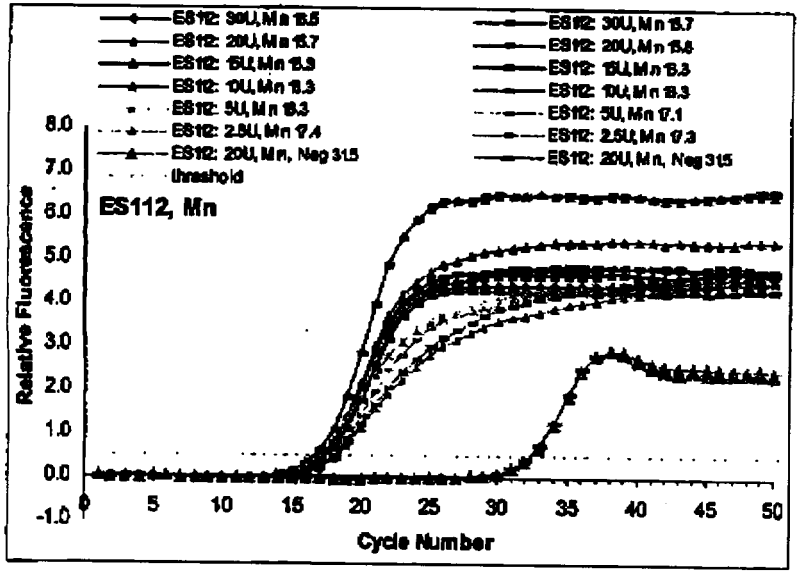


Following a 30 min RT incubation time and Mg^{2+} activation, the mutant enzymes ES112 and ES113 achieved RT activity similar to Mn^{2+}-activated wild-type ZO5 DNA polymerase. With Mn^{2+} activation, the mutant enzymes exhibited similar RT activity, but with much shorter RT incubation times (as low as 5 min). Even with no added RT incubation time there were only slight C_T delays for Mn^{2+}-activated mutant enzyme amplifications and initial PCR ramp times apparently are sufficient for the RT step to occur.

Efficient RT-PCR at Decreased ES112 & ES113 Enzyme Concentrations

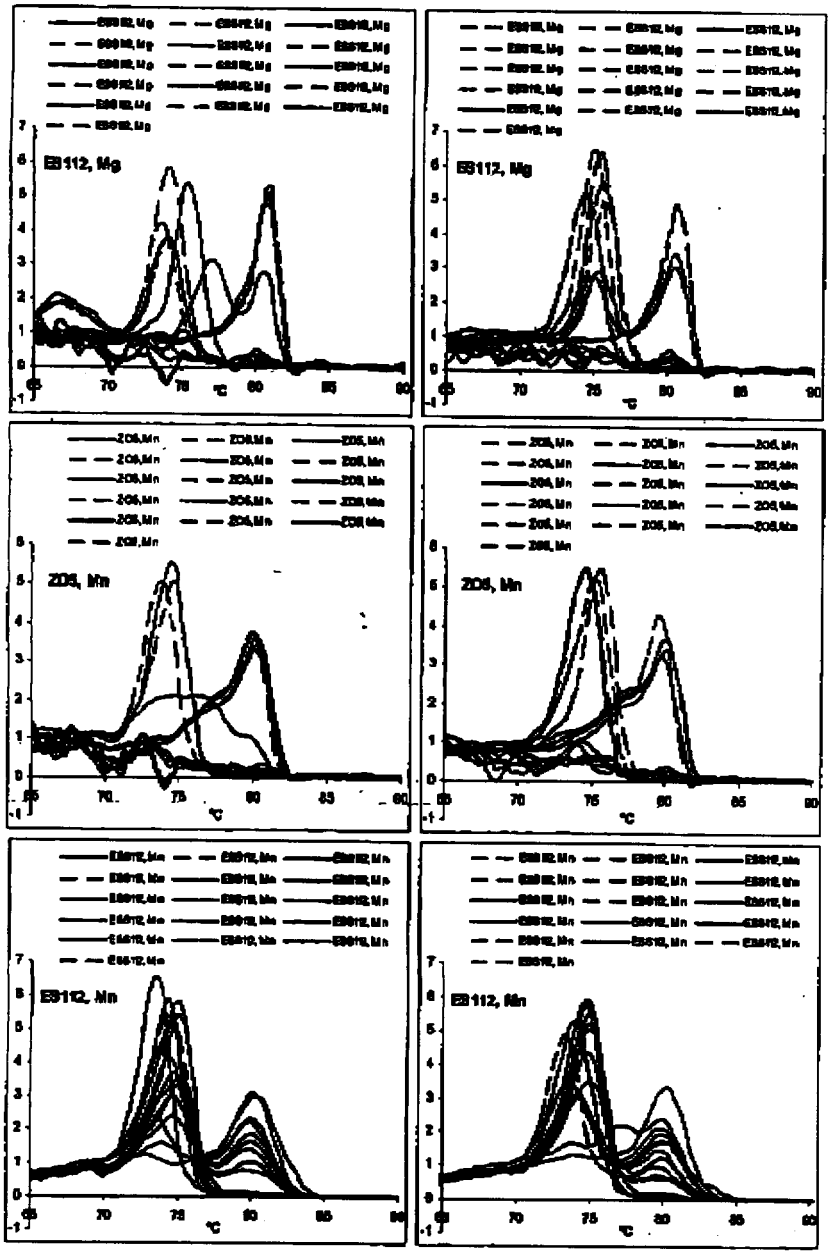






Enzyme concentration was titrated from 30 U down to 2.5 U per reaction for ZO5, ES112 and ES113. A significantly higher C_T value is observed with 2.5 U of ZO5 when compared to higher enzyme concentrations. The ES112 and ES113 perform relatively efficient RT-PCR with as little as 2.5 U of enzyme per 50 µL reaction.

Improved Low Copy Sensitivity with ES112 in Mn^{2+}-activated RT-PCR



ES112, Mg^{2+}

10/32 Positives

ZO5, Mn^{2+}

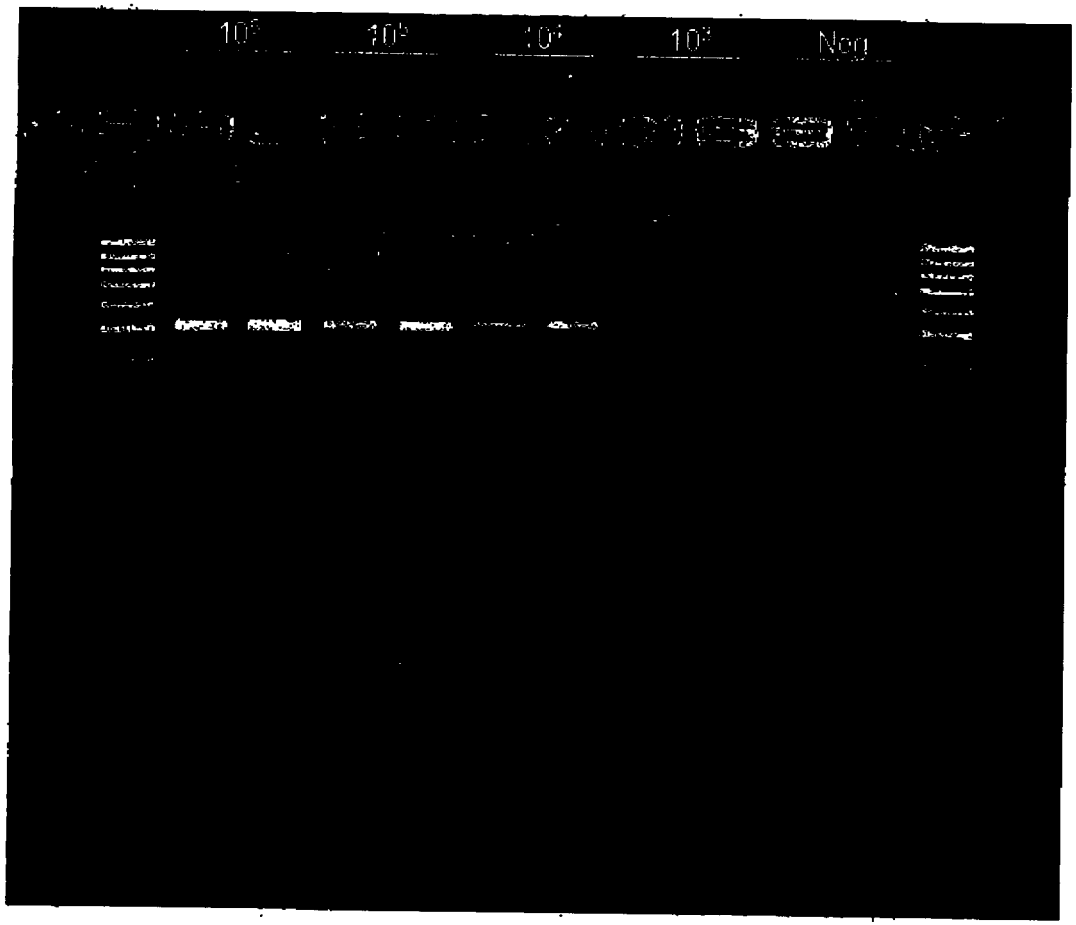
10/32 Positives

ES112, Mn^{2+}

20/32 Positives

Nominally 0.5 copies of HIV transcript RNA per reaction were amplified in 50 µL RT-PCR amplifications optimized for Mg^{2+}-activated ES112, Mn^{2+}-activated ES112 or Mn^{2+}-activated ZO5 ("Gold Standard"). The T_m of end-point RT-PCR product was used to distinguish successful amplification of transcript RNA (specific product) from negative reactions (nonspecific product). The Mg^{2+}-activated ES112 reactions had the same low copy sensitivity as the Mn^{2+}-activated ZO5, while the low copy sensitivity was observed to be twice as good with Mn^{2+}-activated ES112.

RT-PCR Using Mg^{2+}-activated CS6 DNA Polymerase



Various concentrations of pAW109 transcript RNA were amplified by single-buffer RT-PCR. All reactions contained 2 mM Mg^{2+} and CS6 DNA polymerase. Following 45 cycles of PCR, products of the correct size were observed with as little as 10^3 copies of RNA per reaction. Negative control reactions lacking RNA transcript produced no specific product of the expected amplicon size.

CURRICULUM VITAE

David H. Gelfand

Personal Statistics

Date of Birth.	June 9, 1944
Place of Birth:	New York, New York

Education

1970 Ph.D.	Biology, University of California, San Diego, La Jolla, California
1966 A.B.	Biology, Brandeis University, Waltham, Massachusetts

Research and Professional Experience

12/91 - Present 12/00 – Present	Director, Program in Core Research Vice President, Discovery Research Roche Molecular Systems, Inc. 1145 Atlantic Avenue Alameda, CA 94501-1145
11/88 - 12/91	Director, Core Technology, PCR Division, Cetus Corporation
3/81 - 12/91	Vice President, Scientific Affairs, Cetus Corporation
1/79 - 3/81	Vice President and Director of Recombinant Molecular Research, Senior Scientist, Cetus Corporation
12/76 - 10/79	Director, Recombinant Molecular Research Cetus Corporation
8/76 - 1/77	Assistant Research Biochemist, University of California at San Francisco San Francisco, CA
Sponsor:	William J. Rutter, Professor
Project:	Isolation, characterization and expression of eucaryotic DNA sequences in bacterial cells.
1/72 - 8/76	Assistant Research Biochemist and Laboratory Manager, University of California at San Francisco, San Francisco, California
Sponsor:	Gordon M. Tomkins, Professor (deceased July 1975)
Project:	Effect of oncogenic viral transformation on the regulation of gene expression in cultured mammalian cells.
	Isolation and characterization of mutants defective in tyrosine aminotransferase activity.
	Construction of hybrid DNA molecules and genetic transformation.
7/70 - 1/72	Research Associate in Biology, University of California at San Diego, La Jolla, CA
Sponsor:	Masaki Hayashi, Associate Professor
Project:	DNA-dependent RNA-directed protein synthesis *in vitro*: temporal control of transcription and translation.

5/70 - 7/70	NIH postdoctoral trainee in Molecular Genetics, University of California at San Diego, La Jolla, California
Sponsor:	Masaki Hayashi, Associate Professor
Project:	Same as above.
10/66 - 5/70	NIH predoctoral trainee in Molecular Genetics, University of California at San Diego, La Jolla, California
Sponsor:	Masaki Hayashi, Associate Professor
Project:	Viral DNA-dependent protein synthesis
7/66 - 10/66	Research Associate in Biology, University of California at San Diego, La Jolla, CA
Sponsor:	Stanley Mills, Professor
Project:	Passive immune kill in HeLA cells *in vitro*.
6/65 - 9/65	Research Assistant in Biochemistry, Brandeis University, Waltham, Massachusetts
Sponsor:	Gordon Sato, Associate Professor
Project:	Mechanism of steroid production and secretion in mouse tumor cells *in vitro*.
6/62 - 9/62	Research Assistant, School of Medicine, University of Michigan, Ann Arbor, Michigan
Sponsor:	Raymond H. Kahn, Professor
Project:	Effect of *Tubercule bacilli* in chick embryonic lung tissue *in vitro*.
6/61 - 9/61	Research Assistant, Department of Biology, New York University, New York, New York
Sponsor:	M. J. Kopac, Professor
Project:	Establishment of primary cell lines of amphibian liver *in vitro*.

Awards and Honors

New York State S.E. Regional Science Fair, First Prize winner, Senior Division Biology and Grand Prize Winner (1962).

New York State Science Fair Finalist Sixth Prize (1962).

Awarded New York State four-year full-tuition scholarship (award not accepted).

Recipient, May 1990, IPO "Distinguished Inventor Award," Senate Office Building.

Memberships

American Association for the Advancement of Science

American Society of Biochemistry and Molecular Biology

American Society of Microbiology

Genetics Society of America

National Science Foundation Scientific Advisory Council (1981-1984)

Department Visiting Committee, Department of Microbiology, University of Texas, Austin (1988-)

Publications

1 **Gelfand, D.H.,** and Hayashi, M. (1969). Electrophoretic characterization of ΦX174-specific proteins. *J. Mol. Biol.*, 44:501-516.

2 **Gelfand, D.H.,** and Hayashi, M. (1969). DNA-dependent RNA-directed protein synthesis *in vitro*, II: Synthesis of a ΦX174 coat protein component. *Proc. Natl. Acad. Sci. USA*, 63:135-137.

3 Bryan, R.N., **Gelfand, D.H.,** and Hayashi, M. (1969). Initiation of SV40 DNA-directed protein synthesis with N-formylmethionine *in vitro*. *Nature*, 224:1019-1021.

4. **Gelfand, D.H.,** and Hayashi, M. (1970). DNA-dependent RNA-directed protein synthesis *in vitro*, IV: Peptide analysis of an *in vitro* and *in vivo* ΦX174 structural protein. *Proc. Natl. Acad. Sci. USA*, 67:13-17.

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7 **Gelfand, D.H.,** and Hayashi, M. (1970). *In vitro* synthesis of a DNA-dependent RNA polymerase coded on Coliphage T7 genome. *Nature*, 228:1162-1165.

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49. Kalman, L.V., and **Gelfand, D.H.** Mutants of *Thermus aquaticus* DNA Polymerase with Altered Nucleotide Discrimination Properties. *submitted.*

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Issued U.S. Patents

1. **Gelfand, D.H.** "Stable High Copy Number Plasmids." U.S. Patent No. 4,631,257 assigned to Cetus Corp. 12/23/86.

2. **Gelfand, D.H.**, Chang, S., and Wong, H.C. "Polypeptide Expression Using a Portable Temperature Sensitive Control Cassette with a Positive Retroregulatory Element." U.S. Patent No. 4,666,848 assigned to Cetus Corp. 5/19/87.

3. **Gelfand, D.H.** and Lawyer, F.C. "A Portable Temperature-Sensitive Control Cassette." U.S. Patent No. 4,711,845 assigned to Cetus Corp. 12/8/87.

4. **Gelfand, D.H.**, Lawyer, F.C., and Stoffel, S. "Universal Dominant Selectable Marker Cassette." U.S. Patent No. 4,784,949 assigned to Cetus Corp. 11/15/88.

5. **Gelfand, D.H.**, Greenfield, L.I., and Lawyer, F.C. "Recombinant Diphtheria Toxin Fragments." U.S. Patent No. 4,830,962 assigned to Cetus Corp. 5/16/89.

6. **Gelfand, D.H.**, Lawyer, F.C., and Stoffel, S. "SV40 Early and RSV Promoters Useful in *Saccharomyces* Expression." U.S. Patent No. 4,870,013 assigned to Cetus Corp. 9/26/89.

7. **Gelfand, D.H.** and Stoffel, S. "Purified Thermostable Enzyme." U.S. Patent No. 4,889,818 assigned to Hoffmann-La Roche, Inc. 12/26/89.

8. Mullis, K.B., Erlich, H.A., **Gelfand, D.H.**, Horn, G., and Saiki, R.K. "Process for Amplifying Detecting, and/or Cloning Nucleic Acid Sequences Using a Thermostable Enzyme." U.S. Patent No. 4,965,188 assigned to Hoffmann-La Roche, Inc. 10/23/90.

9. **Gelfand, D.H.** "Stable High Copy Number Plasmids." U.S. Patent No. 4,966,840 assigned to Cetus Corp. 10/30/90.

10. Innis, M.A., **Gelfand, D.H.**, and Meade, J.H. "DNA Expression Vector and Use Thereof." U.S. Patent No. 5,045,463 assigned to Cetus Corp. 9/3/91.

11. Innis, M.A., Myambo, K.B., **Gelfand, D.H.**, and Brow, M.A.D. "Methods for DNA Sequencing with *Thermus aquaticus* DNA Polymerase." U.S. Patent No. 5,075,216 assigned to Hoffmann-La Roche, Inc. 12/24/91.

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